RESEARCH NOTE

Clinical epidemiology of ciprofloxacin-resistant *Proteus mirabilis* isolated from urine samples of hospitalised patients

R. Saito[1], S. Okugawa[1], W. Kumita[2], K. Sato[2], T. Chida[2], N. Okamura[2], K. Moriya[1] and K. Koike[1]

[1]Department of Infection Control and Prevention, The University of Tokyo Hospital and [2]Department of Microbiology and Immunology, Graduate School of Health Sciences, Tokyo Medical and Dental University, Tokyo, Japan

ABSTRACT

This study investigated the clinical characteristics of ciprofloxacin-resistant *Proteus mirabilis* isolates from urine samples associated with nosocomial infection or colonisation, and identified the risk-factors for ciprofloxacin resistance. Data for patients with ciprofloxacin-resistant *P. mirabilis* isolates (n = 13) were compared with those for randomly selected patients with ciprofloxacin-susceptible *P. mirabilis* isolates (n = 40) who were matched by temporal occurrence as control patients. The majority of ciprofloxacin-resistant *P. mirabilis* isolates were multiresistant, and ciprofloxacin resistance was associated significantly with previous use of fluoroquinolones and production of extended-spectrum β-lactamases.

Keywords Epidemiology, extended-spectrum β-lactamases, fluoroquinolones, *Proteus mirabilis*, risk-factors, urinary tract infection

Original Submission: 13 April 2007; **Revised Submission:** 22 June 2007; **Accepted:** 11 July 2007

Clin Microbiol Infect 2007; **13:** 1204–1206
10.1111/j.1469-0691.2007.01826.x

Fluoroquinolones are potent antimicrobial agents that are used in the treatment of a wide range of community-acquired and nosocomial infections. However, resistance to fluoroquinolones has increased significantly since the late 1980s [1], and it has been demonstrated that use of fluoroquinolones is an important risk-factor for ciprofloxacin resistance in *Escherichia coli* and *Klebsiella pneumoniae* [2,3]. In addition, following the worldwide use of broad-spectrum cephalosporins during the past two decades, Gram-negative bacteria producing extended-spectrum β-lactamases (ESBLs) have emerged. This emerging resistance is of great concern because its association with resistance to other unrelated antimicrobial agents severely limits therapeutic options [2].

Proteus mirabilis is one of the most common causes of urinary tract infection, and infections caused by this organism are often nosocomial, persistent and difficult to treat. Although wild-type strains of *P. mirabilis* are usually susceptible to fluoroquinolones and β-lactams, a progressive increase in resistance to fluoroquinolones and broad-spectrum cephalosporins has been seen in clinical isolates of this species [4–6]. The relationship between fluoroquinolone resistance and ESBL production in *E. coli* and *K. pneumoniae* is now well-known [3,7,8], but the epidemiology of fluoroquinolone resistance and its relationship with ESBL production in *P. mirabilis* has not yet been clarified. The present study therefore investigated the clinical characteristics of ciprofloxacin-resistant *P. mirabilis* isolates from urine and identified the risk-factors for ciprofloxacin resistance.

The study was conducted at a 1193-bed teaching hospital, providing care for up to 1 million individuals, between April 2003 and March 2006. The medical records of patients were reviewed retrospectively, and all patients with *P. mirabilis* isolated from urine were enrolled in the study. An infectious disease physician evaluated all patients and classified the *P. mirabilis* isolates according to CDC criteria for nosocomial infection [9]. Colonisation was defined when these criteria were not fulfilled. Analysis results for consecutive urine samples containing $\geq 10^5$ CFU/mL with pyuria (≥10 leukocytes/high-power field) were obtained from medical records. Data were also obtained for patients with ciprofloxacin-resistant *P. mirabilis* isolates and for randomly selected patients with ciprofloxacin-susceptible *P. mirabilis* isolates, who were matched by temporal occurrence as control

Corresponding author and reprint requests: R. Saito, Department of Infection Control and Prevention, The University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
E-mail: saito-lab@h.u-tokyo.ac.jp

patients. Data recorded were age, gender, underlying disease, date of hospitalisation, presence of a urinary catheter or a central venous catheter, and antibiotic therapy within the preceding 90 days.

All isolates were identified using the Vitek I system (bioMérieux, Tokyo, Japan). Susceptibility to ciprofloxacin, levofloxacin, minocycline, ampicillin, ampicillin–sulbactam, piperacillin, ceftazidime, cefotaxime, aztreonam, amikacin, gentamicin, imipenem and meropenem was tested using panels manufactured by Eiken Chemical (Tokyo, Japan). Nalidixic acid (Wako Pure Chemical Industries, Osaka, Japan) was also used. MICs were determined by broth microdilution [10]. Quality control organisms for susceptibility tests were *Staphylococcus aureus* ATCC 21293, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. ESBL production was initially detected using cefotaxime and cefotaxime–clavulanate disks, with ESBL-producing *P. mirabilis* isolates being further screened for the presence of class A β-lactamase genes (bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$) by PCR using the primer sets described previously [11,12]. Purified PCR fragments were sequenced using an ABI Prism 310 DNA sequencer (Applied Biosystems, Foster City, CA, USA), followed by a similarity search using the BLAST program (DDBJ, Shizuoka, Japan). Clonal relationships among ciprofloxacin-resistant *P. mirabilis* isolates were investigated by pulsed-field gel electrophoresis following digestion of chromosomal DNA using *Not*I (Roche Diagnostics, Tokyo, Japan). Data were analysed using JMP software, v.6.0 (SAS Institute, Tokyo, Japan) and categorical variables were compared by means of either the chi-square test or Fisher's exact test, as appropriate. A two-tailed p value of <0.05 was considered to be significant.

Among the 80 non-repetitive *P. mirabilis* isolates from urine samples, 13 (16%) were ciprofloxacin-resistant. Of the 13 patients involved, three (23%) were classified as being infected, compared with seven (18%) of 40 control patients (not significant). The antibiotic susceptibilities of the *P. mirabilis* isolates are shown in Table 1. The ciprofloxacin-resistant isolates showed a significantly higher frequency of resistance to ampicillin, piperacillin, ampicillin–sulbactam, cefotaxime, nalidixic acid and levofloxacin (p <0.001). Univariate analysis revealed that previous use of fluoroquinolones (p 0.042) and ESBL production (p <0.001) were associated significantly with ciprofloxacin resistance (Table 1). Eight isolates produced a CTX-M-2 ESBL. Eleven genotypes were identified by pulsed-field gel electrophoresis among the 13 ciprofloxacin-resistant *P. mirabilis* isolates, with three isolates being clonally related.

Table 1. Antibiotic suceptibilities and risk-factors for *Proteus mirabilis* isolates from 13 patients (cases) with ciprofloxacin-resistant isolates and 40 patients (controls) with ciprofloxacin-susceptible isolates

	Cases (n = 13)	Controls (n = 40)	p
Antibiotic resistance frequency, *n* (%)			
Ampicillin	11 (85)	1 (3)	<0.001
Piperacillin	8 (62)	0	<0.001
Ampicillin–sulbactam	11 (85)	0	<0.001
Ceftazidime	0	0	NS
Cefotaxime	8 (62)	0	<0.001
Aztreonam	1 (8)	0	NS
Gentamicin	0	0	NS
Amikacin	0	0	NS
Imipenem	0	0	NS
Meropenem	0	0	NS
Minocycline	11 (85)	25 (63)	NS
Nalidixic acid	13 (100)	9 (23)	<0.001
Levofloxacin	8 (62)	0	<0.001
Risk-factors, *n* (%)			
Age, median years (range)	73 (53–91)	65 (3–94)	NS
Male	7 (54)	14 (35)	NS
Diabetes mellitus	3 (23)	9 (23)	NS
Urinary catheter	6 (46)	18 (45)	NS
Central venous catheter	3 (23)	5 (13)	NS
Long hospital stay (>2 weeks)	5 (38)	14 (35)	NS
ESBL production	8 (62)	0	<0.001
Use of other antibiotics within the previous 90 days	7 (54)	12 (30)	NS
Cephems	1 (8)	8 (20)	NS
Penicillins	2 (15)	1 (3)	NS
Aminoglycosides	1 (8)	1 (3)	NS
Fluoroquinolones	3 (23)	1 (3)	0.042
Carbapenems	2 (15)	2 (5)	NS

ESBL, extended-spectrum β-lactamase; NS, not significant.

Infections caused by fluoroquinolone-resistant ESBL-producing *E. coli* and *K. pneumoniae* strains have now been reported throughout the world. It has also been demonstrated that 18–56% of ESBL-producing *E. coli* and *K. pneumoniae* strains are resistant to fluoroquinolones, and that fluoroquinolone resistance is associated closely with ESBL production [3,7,8]. Another cause for concern is a marked increase in the incidence of *P. mirabilis* isolates that are resistant to fluoroquinolones and broad-spectrum cephalosporins [4–6]. Previous studies have demonstrated that ESBL-producing isolates are usually resistant to penicillins, cephalosporins, aminoglycosides and trimethoprim–sulphamethoxazole [3,6]. The present study revealed that the majority of ciprofloxacin-resistant *P. mirabilis* isolates from urine samples were multiresistant, with most being resistant to cefotaxime and levofloxacin, but not to aminoglycosides.

In the present study, acquisition of ciprofloxacin resistance by *P. mirabilis* was found to be associated significantly with previous use of fluoroquinolones and ESBL production, as was also found for ESBL-producing *E. coli* and *K. pneumoniae* [3,7,8]. While the basis for combined ciprofloxacin and broad-spectrum cephalosporin resistance is not yet fully understood, the presence of both ciprofloxacin resistance and ESBL production may be a consequence of the interplay of previous heavy antibiotic use and conditions that favour patient-to-patient transfer of multidrug-resistant organisms [3]. Other possible explanations include active efflux, outer-membrane alterations and ciprofloxacin resistance mediated by plasmids containing a class A β-lactamase gene.

In conclusion, the present study has certain limitations, in that it was retrospective and sample numbers were small. However, the ciprofloxacin-resistant *P. mirabilis* isolates also exhibited multiresistance, and an analysis of risk-factors showed that previous use of fluoroquinolones and ESBL production were the strongest determinants of the acquisition of ciprofloxacin resistance by *P. mirabilis*. Therefore, when treatment protocols are designed for patients following the isolation of ciprofloxacin-resistant *P. mirabilis* from urine samples, the prevalence of ESBL-producing strains should be taken into consideration, and empirical antibiotic treatments with greater efficacy should be selected accordingly.

REFERENCES

1. Richard P, Delangle MH, Raffi F *et al.* Impact of fluoroquinolone administration on the emergence of fluoroquinolone-resistant gram-negative bacilli from gastrointestinal flora. *Clin Infect Dis* 2001; **32**: 162–166.
2. Hyle EP, Lipworth AD, Zaoutis TE *et al.* Risk factors for increasing multidrug resistance among extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella* species. *Clin Infect Dis* 2005; **40**: 1317–1324.
3. Paterson DL, Mulazimoglu L, Casellas JM *et al.* Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin Infect Dis* 2000; **30**: 473–478.
4. Hernandez JR, Martinez-Martinez L, Pascual A *et al.* Trends in the susceptibilities of *Proteus mirabilis* isolates to quinolones. *J Antimicrob Chemother* 2000; **45**: 407–408.
5. Endimiani A, Luzzaro F, Brigante G *et al. Proteus mirabilis* bloodstream infections: risk factors and treatment outcome related to the expression of extended-spectrum beta-lactamases. *Antimicrob Agents Chemother* 2005; **49**: 2598–2605.
6. Kim JY, Park YJ, Kim SI *et al.* Nosocomial outbreak by *Proteus mirabilis* producing extended-spectrum beta-lactamase VEB-1 in a Korean university hospital. *J Antimicrob Chemother* 2004; **54**: 1144–1147.
7. Lautenbach E, Strom BL, Bilker WB *et al.* Epidemiological investigation of fluoroquinolone resistance in infections due to extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. *Clin Infect Dis* 2001; **33**: 1288–1294.
8. Tolun V, Kucukbasmaci O, Torumkuney-Akbulut D *et al.* Relationship between ciprofloxacin resistance and extended-spectrum beta-lactamase production in *Escherichia coli* and *Klebsiella pneumoniae* strains. *Clin Microbiol Infect* 2004; **10**: 72–75.
9. Garner JS, Jarvis WR, Emori TG *et al*. CDC definitions for nosocomial infections. *Am J Infect Control* 1988; **16**: 128–140.
10. Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, approved standard M7-A7. Wayne, PA: CLSI, 2006.
11. Yagi T, Kurokawa H, Shibata N *et al*. A preliminary survey of extended-spectrum β-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Japan. *FEMS Microbiol Lett* 2000; **184**: 53–56.
12. Pitout JD, Hossain A, Hanson ND. Phenotypic and molecular detection of CTX-M-β-lactamases produced by *Escherichia coli* and *Klebsiella* spp. *J Clin Microbiol* 2004; **42**: 5715–5721.